International Journal of Infectious Diseases (2009) **13**, e420—e423

http://intl.elsevierhealth.com/journals/ijid

Disseminated bacille Calmette—Guérin in Iranian children with severe combined immunodeficiency

Mahnaz Sadeghi-Shabestari [a,*], **Nima Rezaei** [b]

[a] *Division of Pediatric Immunology and Allergy, Children's Hospital, Tabriz University (Medical Sciences), Tabriz, Iran*
[b] *Growth and Development Research Center, Center of Excellence for Pediatrics, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran*

Received 10 February 2008; received in revised form 7 October 2008; accepted 13 February 2009
Corresponding Editor: William Cameron, Ottawa, Canada

KEYWORDS
BCG vaccine;
Disseminated BCG;
Severe combined immunodeficiency

Summary
Background: The bacille Calmette—Guérin (BCG) vaccine is a widely practiced vaccine, which is useful for prophylaxis against tuberculosis. Disseminated BCG infection (BCG-osis) is one of the most important complications of this vaccine and can be seen in patients with an underlying immunodeficiency. This study was performed to determine the underlying defects in patients with BCG-osis.
Methods: Immunological evaluation was performed in all children who developed BCG-osis after vaccination in Tabriz, Iran.
Results: BCG-osis was documented in eight patients following vaccination. Axillary adenitis was detected in seven patients, and hepatosplenomegaly was also found in seven patients. Immunological studies revealed low serum IgG, IgM and IgA levels in all patients. Further investigations for enumeration of the lymphocyte sub-population revealed severe combined immunodeficiency (SCID) in all the patients. Three patients had T—B+NK— SCID, four had T—B—NK+ SCID, and one had T—B+NK+ SCID. Unfortunately, all the patients died due to the severe disseminated BCG infection.
Conclusions: Inoculation of live vaccines such as BCG should be postponed for a few months in suspected cases of primary immunodeficiency disease, until appropriate screening tests exclude this diagnosis; vaccination should then be performed in those with an intact immune system.

Introduction

The bacille Calmette—Guérin (BCG) vaccine is widely practiced in many countries for prophylaxis against tuberculosis (TB), especially in the pediatric age group. Although it is considered safe in general, there are several complications, ranging from regional disease (BCG-itis) to disseminated disease (BCG-osis). However, BCG-osis, which is associated with a high mortality rate, is very rare. This BCG complication may suggest an underlying immunodeficiency.[1—6]

Primary immunodeficiency diseases (PID) are a heterogeneous group of disorders, characterized by an increased susceptibility to infections.[7,8] It appears that a number of PID result in an increased susceptibility to severe mycobacterial disease following vaccination with BCG, including

* Corresponding author. Tel.: +98 411 334 0533; fax: +98 411 526 2280.
E-mail address: drsadeghim2004@yahoo.com (M. Sadeghi-Shabestari).

1201-9712/$36.00
doi:10.1016/j.ijid.2009.02.008

severe combined immunodeficiency (SCID), chronic granulomatous disease (CGD), and Mendelian susceptibility to mycobacterial diseases (e.g., IFN-γ receptor 1/2 deficiencies, IL-12/23 receptor β1 chain deficiency, IL-12p40 deficiency, STAT1 deficiency, LZ-NEMO deficiency).[8–15]

This study was performed in order to determine the underlying defects in patients referred to the Children's Hospital due to BCG-osis.

Patients and methods

This was a prospective study on children who developed disseminated BCG after vaccination in Tabriz (East Azerbaijan, Iran). The study was approved by the local ethics committee of the Tabriz Children's Hospital. Eight patients, who had BCG-osis, were referred to the Tabriz Children's Hospital, the main referral hospital in Tabriz, and an immunological evaluation was performed in all these patients.

All of the study patients had been inoculated with the BCG vaccine at birth, and clinical and laboratory findings of the patients were compatible with a diagnosis of disseminated BCG. They had two or more signs and symptoms of a systemic syndrome compatible with mycobacterial disease, including fever, weight loss, lymphadenopathy, cutaneous abscesses, pneumonia, osteomyelitis, hepatomegaly, and splenomegaly. Evidence of BCG infection was also confirmed by histopathologic findings of acid-fast bacilli at two or more anatomic sites (e.g., lymph nodes) outside the region of vaccination, in gastric aspiration or in bone marrow aspiration.[5]

After taking the medical history and physical examination, further laboratory investigations were performed to determine the underlying defects. Consanguineous marriage was defined as partners who have at least one ancestor in common, with the ancestor being no more distant than a great great grandparent. For descendants of the same generation, a consanguineous marriage would be between one person and a third cousin or a closer relative.[16]

In these patients with confirmed disseminated BCG, screening laboratory tests, including complete blood count and differential count (platelet volume, absolute lymphocyte count, neutrophil and eosinophil counts), tests for HIV, and serum immunoglobulin levels (IgG, IgM and IgA) were evaluated. As the results suggested SCID, lymphocyte subpopulations including CD19+ B-cells, CD3+ T-cells, CD56+ natural killer (NK) cells, and also CD3+CD4+ and CD3+CD8+ T-cells were measured by double staining of lysed whole blood followed by flow cytometric analysis. Other screening tests (e.g., nitroblue tetrazolium (NBT) for CGD) were not done as the diagnosis of SCID had been confirmed at this point.

SCID was sub-classified based on the different peripheral lymphocyte immunophenotyping as follows: T−B−NK+ SCID (absent circulating T- and B-cells, but NK cells present), T−B+NK− (absent circulating T- and NK cells, but B-cells present), and T−B+NK+ (absent circulating T-cells, but B- and NK cells present).[6,12]

Results

Disseminated BCG infection was documented in eight patients (four male and four female) following routine BCG immunization. All patients were vaccinated at birth and were subsequently complicated with BCG-osis. The parents of six cases were consanguine. A family history of early onset severe infections leading to death in infancy was documented in three families (Table 1). As shown in Figure 1, the pedigree of P1 shows the family of a SCID patient whose parents were consanguine. There was a history of stillbirth in this family, but evaluation for SCID had not been performed for the stillbirth. The pedigree of P2 shows the family of a SCID patient from non-consanguine parents. There was history of early death due to severe infections in his sibling and his cousin, but evaluation for SCID had not been performed for those cases.

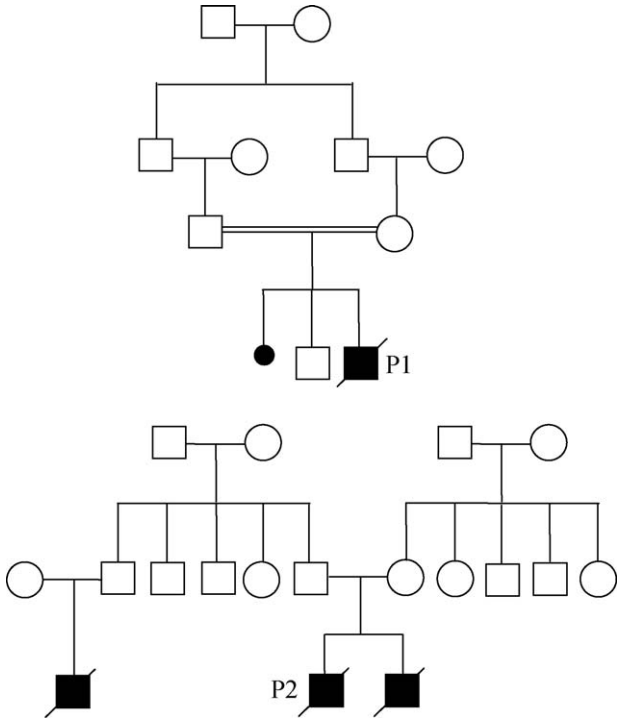

Figure 1 The pedigrees of two families with disseminated BCG and severe combined immunodeficiency (open shapes represent healthy individuals, filled shapes represent affected patients, small filled shapes represent stillbirth, and shapes with slashes represent deceased individuals; boxes: males, circles: females).

Axillary adenitis was detected in seven cases (all except P2), while seven patients (all except P4) had hepatosplenomegaly at the time of admission. In addition to BCG infection, seven cases (all except P3) suffered from candidiasis; P2 and P8 had diffuse candidiasis and the others had oral candidiasis.

Acid-fast bacilli were detected in the lymph nodes of seven patients, all except P2 who did not have lymphadenopathy. Seven cases experienced recurrent diarrhea. Gastric aspiration also showed acid-fast bacilli in patients P1, P2, P3 and P8. Two patients (P2 and P4) had arthritis; patient P4 had tuberculosis osteitis. Bone marrow aspiration also demonstrated acid-fast bacilli. Moreover four cases experienced severe pneumonia (P1, P4, P6, P7), but specific organisms were not identified. Patient P4 also presented with pleural effusion and patient P6 was complicated with eye involvement, which led to blindness.

Immunoglobulin serum levels were measured as the first screening test for PID. All had low serum IgG, IgM and IgA.

Table 1 Characteristics of the patients with disseminated BCG

Number	Sex	Diagnosis age (months)	Age at death (months)	Consanguinity of parents	Family history of recurrent infections
P1	Male	4	6	Yes	No
P2	Male	4	4	No	Yes
P3	Female	5	5	No	No
P4	Female	5	5	Yes	Yes
P5	Female	5	7	Yes	Yes
P6	Male	4	4	Yes	No
P7	Female	4	4	Yes	No
P8	Male	6	7	Yes	No

BCG, bacille Calmette–Guérin.

Table 2 Laboratory investigations in the patients with disseminated BCG

Number	IgG (mg/dl)	IgM (mg/dl)	IgA (mg/dl)	WBC count ($\times 10^9$ cells/l)	Lymphocyte count ($\times 10^9$ cells/l)	Absolute count (cells/mm^3)					SCID diagnosis
						CD19	CD3	CD4	CD8	CD56	
P1	29	9	8	5.7	0.627 (11%)	31	31	25	113	439	T−B−NK+
P2	70	23	11	9.5	0.570 (6%)	143	11	11	34	0	T−B+NK−
P3	180	270	13	6.2	1.488 (24%)	1220	30	15	30	60	T−B+NK−
P4	0	0	0	5.2	1.404 (27%)	0	154	112	42	491	T−B−NK+
P5	220	29	8	9.9	4.158 (42%)	1455	1122	499	624	499	T−B+NK+
P6	211	34	26	5.94	2.079 (35%)	83	520	83	478	1309	T−B−NK+
P7	181	31	36	9.8	0.323 (3.3%)	55	13	10	16	191	T−B−NK+
P8	50	15	0	8.3	3.652 (44%)	1169	1460	475	584	183	T−B+NK−

BCG, bacille Calmette–Guérin; WBC, white blood cell; SCID, severe combined immunodeficiency; T, T-cell; B, B-cell; NK, natural killer cell.

Further investigations for the enumeration of lymphocyte sub-populations revealed SCID in all these patients (Table 2). Among them, three had T−B+NK− SCID, four had T−B−NK+ SCID, and one had T−B+NK+ SCID (Table 2).

Unfortunately, all the patients died due to severe disseminated BCG infection.

Discussion

In this study, all patients with BCG-osis had SCID. BCG-osis is one of the most common causes of death in PID patients, particularly in those with SCID.[8] SCID is a heterogeneous group of diseases that affects cellular and humoral immune function and can be categorized into different groups with different underlying genetic defects.[7] Based on the presence of B-cells, SCID can be categorized into two groups, T−B+ SCID and T−B− SCID. These can subsequently be divided into two subgroups based on the presence of NK cells.

The underlying genetic defects in SCID are not always reported, but it appears that all types of SCID are susceptible to BCG.[13] Although molecular studies were not performed in these patients, γc deficiency (P3 and P8) and JAK3 deficiency (P2) could be suspected in the patients with T−B+NK− SCID, according to the associated phenotypic abnormalities. CD3, CD45 and IL7-Rα deficiencies could be suspected in the patient with T−B+NK+ SCID (P5), while RAG1/2 and Artemis deficiencies could be suspected in the patients with T−B−NK+ SCID (P1, P4, P6 and P7).

Apart from the type of SCID and the underlying genetic defect, patients with SCID are susceptible to the majority of microorganisms and life-threatening opportunistic infections in early infancy.[10,13,17] Several cases with SCID develop complications after BCG vaccination, with BCG-osis and even death occurring in some of them.[9,17,18]

It is commonly advised that the first dose of BCG vaccine be administered at birth, particularly in regions with a higher incidence of TB. It is estimated that the incidence of new smear-positive tuberculosis in Iran is 24 per 100 000 population countrywide,[19] while the notification rate increases up to 135 per 100 000 population in eastern Iran, neighboring Afghanistan.[20] Although the vaccine can prevent severe and disseminated forms of tuberculosis, such as meningitis and miliary TB, there is some controversy surrounding its protective efficacy against pulmonary TB.[21]

There was a positive family history of early death due to recurrent infections in some patients, which should be considered an alarm for PID. However, properly assessing for family history of neonatal deaths and recurrent infections is not easy for front-line healthcare providers, and there are inherent difficulties in relying on history. Consanguinity is another important issue in the family history that was reported in six families in this study; the resulting children are prone to autosomal recessive congenital disorders. The rate of consanguineous marriage in Iranian SCID patients is more than 80%, much higher than the rate in the normal population and also in other PID.[8] Due to this high consanguinity rate in the region, providing facilities for genetic counseling and

reproductive risk assessment, as well as public education programs are recommended.[16]

While the BCG vaccine is routinely given to all Iranian children at birth, this vaccine should be prohibited in some specific PID cases, especially SCID, and also in their families who may have PID.[11] Considering the high incidence of SCID in northwestern Iran,[8,22] delaying BCG vaccination for a few months could be suggested in those families with a history of recurrent infections or early death; however, vaccination should definitely be performed later in those cases with an intact immune system. Following this policy, several SCID patients will not be vaccinated with BCG, while several cases will still be missed. With regard to the difficulty in implementing such a guideline in settings where BCG is given to all newborns, changing the worldwide policy, or at least the regional program of BCG vaccination from birth to 4 or 6 months could help healthcare workers to investigate underlying disease; SCID patients usually become symptomatic during this period. However, a further worldwide survey is needed to make the best decision on this issue.

Unfortunately, the prognosis for children with SCID is very poor in Iran. Recent reports indicate that all Iranian SCID patients die during the first year of life.[8,10] Stem cell transplantation is the only curative treatment in this group of patients, however there are only a few centers for stem cell transplantation in Iran, and these do not have enough experience to undertake transplantation in primary immunodeficiency diseases. Delay in diagnosis and late referral of patients is another reason why stem cell transplantation has not been successful in the country.[8]

SCID patients have an increased susceptibility to mycobacterium infection and primarily to BCG vaccine.[13] It is recommended that inoculation of live vaccines such as BCG should be postponed in those newborns of families with a history of recurrent infections or early death, until appropriate screening tests exclude a diagnosis of PID. Further epidemiological studies are needed to estimate the rate of disseminated BCG infection in the region, while worldwide studies on disseminated BCG and SCID may be worthwhile in order to prepare guidelines on this topic.

Conflict of interest: No conflict of interest to declare.

References

1. Casanova JL, Jouanguy E, Lamhamedi S, Blanche S, Fischer A. Immunological conditions of children with BCG disseminated infection. *Lancet* 1995;**346**:581.
2. Cohn DL. Use of the bacille Calmette—Guérin vaccination for the prevention of tuberculosis: renewed interest in an old vaccine. *Am J Med Sci* 1997;**313**:372—6.
3. Gonzalez B, Moreno S, Burdach R, Valenzuela MT, Henriquez A, Ramos MI, et al. Clinical presentation of Bacillus Calmette—Guérin infections in patients with immunodeficiency syndromes. *Pediatr Infect Dis J* 1989;**8**:201—6.
4. Casanova JL, Blanche S, Emile JF, Jouanguy E, Lamhamedi S, Altare F, et al. Idiopathic disseminated bacillus Calmette—Guérin infection: a French national retrospective study. *Pediatrics* 1996;**98**:774—8.
5. Afshar Paiman S, Siadati A, Mamishi S, Tabatabaie P, Khotaee G. Disseminated *Mycobacterium bovis* infection after BCG vaccination. *Iran J Allergy Asthma Immunol* 2006;**5**:133—7.
6. Deeks SL, Clark M, Scheifele DW, Law BJ, Dawar M, Ahmadipour N, et al. Serious adverse events associated with bacille Calmette—Guérin vaccine in Canada. *Pediatr Infect Dis J* 2005;**24**:538—41.
7. Geha RS, Notarangelo L, Casanova JL, Chapel H, Fischer A, Hammarstrom L, et al. Primary immunodeficiency diseases: an update the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee. *J Allergy Clin Immunol* 2007;**120**:776—94.
8. Rezaei N, Aghamohammadi A, Moin M, Pourpak Z, Movahedi M, Gharagozlou M, et al. Frequency and clinical manifestations of patients with primary immunodeficiency disorders in Iran: update from the Iranian Primary Immunodeficiency Registry. *J Clin Immunol* 2006;**26**:519—32.
9. Huang LH, Shyur SD, Weng JD, Shin-Chi. Huang FY, Tzen CY. Disseminated cutaneous bacille Calmette—Guérin infection identified by polymerase chain reaction in a patient with X-linked severe combined immunodeficiency. *Pediatr Dermatol* 2006;**23**:560—3.
10. Yeganeh M, Heidarzade M, Pourpak Z, Parvaneh N, Rezaei N, Aghamohammadi A, et al. Severe combined immunodeficiency: a cohort of forty patients. *Pediatr Allergy Immunol* 2008;**19**:303—6.
11. Movahedi M, Aghamohammadi A, Rezaei N, Shahnavaz N, Jandaghi AB, Farhoudi A, et al. Chronic granulomatous disease: a clinical survey of 41 patients from the Iranian primary immunodeficiency registry. *Int Arch Allergy Immunol* 2004;**134**:253—9.
12. Bustamante J, Aksu G, Vogt G, de Beaucoudrey L, Genel F, Chapgier A, et al. BCG-osis and tuberculosis in a child with chronic granulomatous disease. *J Allergy Clin Immunol* 2007;**120**:32—8.
13. Reichenbach J, Rosenzweig S, Doffinger R, Dupuis S, Holland SM, Casanova JL. Mycobacterial diseases in primary immunodeficiencies. *Curr Opin Allergy Clin Immunol* 2001;**1**:503—11.
14. Filipe Santos O, Bustamante J, Chapgier A, Vogt G, de Beaucoudrey L, Feinberg J, et al. Inborn errors of the IL-12/23 and IFN-gamma mediated immunity: molecular, cellular and clinical features. *Semin Immunol* 2006;**18**:347—61.
15. Moin M, Farhoudi A, Movahedi M, Rezaei N, Pourpak Z, Yeganeh M. The clinical and laboratory survey of Iranian patients with hyper-IgE syndrome. *Scand J Infect Dis* 2006;**38**:898—903.
16. Rezaei N, Pourpak Z, Aghamohammadi A, Farhoudi A, Movahedi M, Gharagozlou M, et al. Consanguinity in primary immunodeficiency disorders; the report from Iranian primary immunodeficiency registry. *Am J Reprod Immunol* 2006;**56**:145—51.
17. Stephan JL, Vlekova V, Le Deist F, Blanche S, Donadieu J, De Saint-Basile G, et al. Severe combined immunodeficiency: a retrospective single-center study of clinical presentation and outcome in 117 patients. *J Pediatr* 1993;**123**:564—72.
18. Abramowsky C, Gonzalez B, Sorensen RU. Disseminated bacillus Calmette—Guérin infections in patients with primary immunodeficiencies. *Am J Clin Pathol* 1993;**100**:52—6.
19. Global tuberculosis control. World Health Organization reports 1996-2002. TB situation in the region: Islamic Republic of Iran. Presented at the Meeting of National Managers of the Tuberculosis Control Programmes in the Eastern Mediterranean Region, Rabat, June 17-19, 2003.
20. Khazaei HA, Rezaei N, Bagheri GR, Dankoub MA, Shahryari K, Tahai A, et al. Epidemiology of tuberculosis in the southeastern Iran. *Eur J Epidemiol* 2005;**20**:879—83.
21. Barreto ML, Pereira SM, Ferreira AA. BCG vaccine: efficacy and indications for vaccination and revaccination. *J Pediatr (Rio J)* 2006;**82**:45—54.
22. Sadeghi Shabestari M, Maljaei SH, Baradaran R, Barzegar M, Hashemi F, Mesri A, et al. Distribution of primary immunodeficiency diseases in the Turk ethnic group, living in the northwestern Iran. *J Clin Immunol* 2007;**27**:510—6.